Prognostic or predictive value of MGMT promoter methylation in gliomas depends on IDH1 mutation

Wick, W ; Meisner, C ; Hentschel, B ; Platten, M ; Schilling, A ; Wiestler, B ; Sabel, M C ; Koeppen, S ; Ketter, R ; Weiler, M ; Tabatabai, G ; von Deimling, A ; Gramatzki, D ; Westphal, M ; Schackert, G ; Loeffler, M ; Simon, M ; Reifenberger, G ; Weller, M

Abstract: OBJECTIVE: To explore whether the isocitrate dehydrogenase 1 (IDH1) or 1p/19q status determines the prognostic vs predictive role of O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in the Neuro-Oncology Working Group of the German Cancer Society (NOA)-04 trial anaplastic glioma biomarker cohort. METHODS: Patients (n = 183) of the NOA-04 trial with known MGMT and IDH1 status were analyzed for interdependency of the prognostic vs predictive role of MGMT promoter methylation from IDH1 or 1p/19q status and treatment, using progression-free survival (PFS) as an endpoint. An independent validation cohort of the German Glioma Network (n = 75) and the NOA-08 trial (n = 34) served as a confirmation cohort. RESULTS: In tumors with IDH1 mutation, MGMT promoter methylation was associated with prolonged PFS with chemotherapy ± radiotherapy (RT) or RT-only groups, and is thus prognostic. In tumors without IDH1 mutation, MGMT promoter methylation was associated with increased PFS in patients treated with chemotherapy, but not in those who received RT alone as the first-line treatment, and is thus chemotherapy-predictive. In contrast, 1p/19q codeletions showed no such association with the prognostic vs predictive value of MGMT. CONCLUSIONS: MGMT promoter methylation is a predictive biomarker for benefit from alkylating agent chemotherapy in patients with IDH1-wild-type, but not IDH1-mutant, malignant gliomas of World Health Organization grades III/IV. Combined IDH1/MGMT assessment may help to individualize clinical decision-making in neuro-oncology.

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Prognostic or predictive value of *MGMT* promoter methylation in gliomas depends on *IDH1* mutation

Wolfgang Wick, MD[1,3], Christoph Meisner, PhD[5], Bettina Hentschel, PhD[6], Michael Platten, MD[1], Alissa Schilling, MD[1], Benedikt Wiestler, MD[1,3], Michael C. Sabel, MD[7], Susanne Koeppen, MD[10], Ralf Ketter, MD[11], Markus Weiler, MD[1,3], Ghazaleh Tabatabai, MD, PhD[12], Andreas von Deimling, MD[2,4], Dorothee Gramatzki, MD[12], Manfred Westphal, MD[13], Gabriele Schackert, MD[14,15], Markus Loeffler, MD[6], Matthias Simon, MD[16], Guido Reifenberger, MD[8,9], Michael Weller, MD[12]

[1]Departments of Neurooncology and [2]Neuropathology, University of Heidelberg, Im Neuenheimer Feld 400, and [3]German Cancer Consortium (DKTK), Clinical Cooperation Unit Neurooncology and [4]Clinical Cooperation Unit Neuropathology, German Cancer Research Center, D-69120 Heidelberg; [5]Department of Medical Biometry, University of Tübingen, Westbahnhofstrasse 55, D-72070 Tübingen; [6]Institute for Medical Informatics, Statistics and Epidemiology (IMISE), University Leipzig, Härtelstrasse 16-18, 04107 Leipzig; [7]Departments of Neurosurgery and [8]Neuropathology, Heinrich-Heine-University and [9]DKTK, Düsseldorf, Moorenstrasse 5, D-40225 Düsseldorf; [10]Department of Neurology, University of Essen Medical School, Hufelandstrasse 55, D-45147 Essen; [11]Department of Neurosurgery, Saarland University, 66421 Homburg, all Germany; [12]Department of Neurology, University Hospital Zurich, Frauenklinikstrasse 26, CH-8091 Zürich, Switzerland; [13]Department of Neurosurgery, University Clinic Hamburg Eppendorf, Martinistr. 52, 20246 Hamburg; [14]Department of Neurosurgery and [15]DKTK, Technical University Dresden, Fetscherstraße 74, 01307 Dresden; [16]Department of Neurosurgery, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, all Germany

Corresponding Author: Dr. Wolfgang Wick, Ruprecht-Karls-Universität, Heidelberg, Department of Neurooncology, Neurology Clinic & National Center for Tumor Disease, Im Neuenheimer Feld 400, D-69120 Heidelberg, phone +49 (0)6221 56 7075, fax +49 (0)6221 56 7554, email: wolfgang.wick@med.uni-heidelberg.de

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Author Contributions

Dr. Wick: conception & design, data analysis, manuscript writing, final approval. Dr. Meisner: data analysis, manuscript writing, final approval. Dr. Hentschel: data analysis, manuscript writing, final approval. Dr. Platten: data acquisition, manuscript writing, final approval. Dr. Schilling: data acquisition, manuscript writing, final approval. Dr. Wiestler: data acquisition, manuscript writing, final approval. Dr. Sabel: data acquisition, manuscript writing, final approval. Dr. Koeppen: data acquisition, manuscript writing, final approval. Dr. Ketter: data acquisition, manuscript writing, final approval. Dr. Weiler: data acquisition, manuscript writing, final approval. Dr. Tabatabai: data acquisition, manuscript writing, final approval. Dr. von Deimling: data acquisition, manuscript writing, final approval. Dr. Gramatzki: data acquisition, manuscript writing, final approval. Dr. Westphal data acquisition, manuscript writing, final approval. Dr. Schackert: data acquisition, manuscript writing, final approval. Dr. Loeffler: data analysis, manuscript writing, final approval. Dr. Simon: data acquisition, manuscript writing, final approval. Dr. Reifenberger: data acquisition, manuscript writing, final approval. Dr. Weller: conception & design, data analysis, manuscript writing, final approval.

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Abstract

Objective

To explore whether the *IDH1* or 1p/19q status determine the prognostic versus predictive role of *MGMT* promoter methylation in the NOA-04 trial anaplastic glioma biomarker cohort.

Methods

Patients (n=183) of the NOA-04 trial with known *MGMT* and *IDH1* status were analyzed for interdependency of the prognostic *versus* predictive role of *MGMT* promoter methylation from *IDH1* or 1p/19q status and treatment, using PFS as an endpoint. An independent validation cohort of the German Glioma Network (GGN) (n=75) and the NOA-08 trial (n=34) served as a confirmation cohort.

Results

In tumors with *IDH1* mutation, *MGMT* promoter methylation was associated with prolonged PFS with chemotherapy ± radiotherapy (RT) or RT only groups, and thus prognostic. In tumors without *IDH1* mutation, *MGMT* promoter methylation was associated with increased PFS in patients treated with chemotherapy, too, but not in those who received RT alone as the first-line treatment, and is thus chemotherapy-predictive. In contrast 1p/19q codeletions showed no such association with the prognostic versus predictive value of *MGMT*.

Conclusions

MGMT promoter methylation is a predictive biomarker for benefit from alkylating agent chemotherapy in patients with *IDH1*-wildtype, but not *IDH1*-mutant malignant gliomas of WHO grades III/IV. Combined *IDH1/ MGMT* assessment may help to individualize clinical decision making in neurooncology.

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Introduction

The predictive power of O^6-methylguanine DNA-methyltransferase (*MGMT*) promoter methylation for benefit from temozolomide (TMZ) as seen in glioblastoma [1,2] was not detected in anaplastic glioma either in the Neurooncology Working Group of the German Cancer Society (NOA)-04 trial[3] or in the anaplastic oligodendroglial tumor EORTC 26951 cohort.[4,5] Here, *MGMT* promoter methylation was similarly prognostic for better outcome with both alkylating chemotherapy and radiotherapy (RT).

Among the explanations for these differences is a confounding influence of *isocitrate dehydrogenase 1* (*IDH1*) mutations, which are associated with a CpG island methylator phenotype in glioma (GCIMP).[6] Mutations in *IDH1* have been identified in approximately 60 to 80% of gliomas of WHO grades II and III and in secondary glioblastomas, but only in approximately 5% of primary glioblastomas.[7,8] Patients with malignant gliomas carrying *IDH1* mutations have a better outcome than patients with *IDH1-w*ildtype gliomas, regardless of the specific treatment.[1,8-12] Primary glioblastomas without *IDH1* mutation are biologically different.[13] Similarly, also *IDH1-w*ildtype low-grade and anaplastic gliomas are prognostically distinct from *IDH1*-mutated grade II/III gliomas. Importantly, the prognostic properties of all markers described so far become apparent only with any form of postoperative genotoxic treatment and do not signify the natural postoperative course of disease.[12,14]

The present analysis was performed to test the hypothesis that the *IDH1* status (mutant *versus* wildtype) rather than histological grading (WHO grade III *versus* IV) determines whether *MGMT* promoter methylation is prognostic for the benefit from either type of genotoxic therapy, RT or chemotherapy, or predictive specifically for benefit from alkylating agent chemotherapy.

Patients and Methods

Patients and evaluations – NOA-04 trial - training cohort

The NOA-04 trial randomized adult patients with histologically confirmed WHO grade III anaplastic glioma to either RT (arm A) or alkylating chemotherapy (arms B/C).[3] Histologic diagnosis of anaplastic glioma was confirmed centrally at the Brain Tumor Reference Center in Bonn before study entry according to WHO classifications 1993[15] and 2000.[16] Median follow-up time was 54 months.

Standard protocol approvals, registrations, and patients' consents

The NOA-04 trial (NCT00717210) was approved by the central ethics committee at the University of Tuebingen (106/99) and all 39 partner sites and enrolled patients after written informed consent, which included molecular analyses performed with study data and materials.

Patients and evaluations – German Glioma Network (GGN) validation cohort

The GGN is a prospective cohort study that enrolled 2,549 newly diagnosed patients with various types of glioma and frozen tissue asservation from October 2004 to October 2010. From this cohort we identified 363 patients with a diagnosis of primary anaplastic astrocytoma (n=75) or glioblastoma (n=288) confirmed by history taking and central pathology review[17], as well as adequate follow-up at least until progression, who were treated with RT alone or alkylator-based chemo- or radiochemotherapy. Two hundred-thirty-nine patients were included in previous publications.[12,18] Clinical data were prospectively documented on CRF and centrally assembled as outlined before (http://www.gliomnetzwerk.de).[12] The patients were not commonly enrolled into clinical trials, and treatment decisions were made by the treating physicians, patients and their families, without awareness of results of molecular parameters. Progression was defined locally at standardized clinical and MRI examinations[19] and not centrally reviewed. All patients gave

written informed consent. The review boards of the participating institutions approved all activities of the GGN.

Patients and evaluations – the NOA-08 anaplastic astrocytoma biomarker validation cohort

The NOA-08 trial randomized elderly patients with malignant astrocytoma to primary RT or TMZ between 2005 and 2009.[20] Patients from this trial with anaplastic astrocytoma as well as information on MGMT[22] and IDH1 status (Table 1) were pooled with the anaplastic glioma cohort of the GGN.

Molecular evaluations

For subpopulations from the NOA-04 and NOA-08 trials as well a the GGN cohort, for which biomaterial was available, *MGMT* promoter methylation (methylation-specific PCR), 1p/19q codeletions (fluorescence *in situ* hybridization) and *IDH* mutations (immunohistochemistry for IDH1 and sequencing for *IDH*1/2) status were assessed according to routine methods.[3,12] The NOA-04 subgroup reported here was representative for the *per protocol* population of the trial (Table e-1).

Statistical analyses

The primary endpoint of NOA-04 was time from surgery to treatment failure (TTF) stratified for therapy in the intention-to-treat (ITT) population. Treatment failure was defined as withdrawal from therapy before progression after chemotherapy and RT in either sequence because of toxicity or poor clinical condition, progression after chemo- and radiotherapy in either sequence, or death.[3] Secondary end points included progression-free survival (PFS), overall survival (OS), and clinical efficacy endpoints (TTF, PFS and OS) stratified for histology, 1p/19q co-deletion, *MGMT* promoter methylation, and *IDH1* mutation status.

Tests used for homogeneity in the NOA-04 biomarker cohort were for age and KPS: Wilcoxon-test; for all other variables: Fisher-Exact-test. Missing values were excluded from the statistical tests. Here, we focused our analysis on PFS since differentiation between

prediction and prognosis was the primary aim of the present *post hoc* analysis. Univariate analysis of PFS used Kaplan-Meier estimates.[21] Multivariate analysis used a Cox proportional hazards model fitted to adjust for confounding variables. Hazard ratios (HR) with 95%-confidence intervals were estimated. The analysis was done in two steps. In the first step, we used the cohort of the NOA-04 trial to generate hypotheses concerning the role of *MGMT* promoter methylation and *IDH1* mutation status as possible prognostic or predictive factors for PFS. In the second step, we aimed at confirming these hypotheses in the independent GGN/NOA-08 cohort. Analyses for interaction between *IDH1* or 1p/19q and *MGMT* status for PFS were done using the Statistical Analysing Programme SAS 9.1.3 and IBM SPSS Statistics Release 20.0.0.

Results

Information on *MGMT* and *IDH1* status was available for 183 NOA-04 patients that was used as a training cohort. This subgroup was comparable to the group of 91 patients without *MGMT* and *IDH1* status data available concerning the distribution of type of primary surgery, histology, age and therapy group. The distribution to the RT arm and to the chemotherapy arms regarding baseline characteristics was balanced (Table 1). Baseline data for the GGN/NOA-08 pooled anaplastic astrocytoma cohort used as a validation cohort commonly treated with RT alone (n=42) or chemotherapy ± RT (n=67) is also provided in Table 1.

In the NOA-04 cohort, PFS was overall longer in patients with *IDH1*-mutant tumors than in patients with *IDH1-w*ildtype tumors (41.6 *versus* 15.2 months, p<0.0001). Also, PFS was longer in patients with *MGMT*-methylated tumors compared with those with *MGMT*-unmethylated tumors (41.6 *versus* 16.9 months, p<0.0001). To answer the question whether the prognostic or therapy modality-predictive impact of *MGMT* promoter methylation depends on the *IDH1* status, we compared PFS in the four groups separated by treatment. Patients with *IDH1*-mutant tumors had a longer PFS when the *MGMT* promoter was methylated, both with RT or chemotherapy. In patients with *IDH1*-wildtype tumors treated with RT, PFS did not differ dependent on the *MGMT* status, but patients without *MGMT* promoter methylation had a dramatically worse PFS when treated with alkylating chemotherapy alone (Table 2). Interestingly, a therapy-specific association was neither found for the 1p/19q co-deleted patients with (n=71) and without (n=7) *IDH1* mutation nor the 1p/19q intact patients with (n=55) and without (n=49) *IDH1* mutation (data not shown). There were no *IDH2* mutations in the samples analyzed. For the time-to-treatment failure, which generally meant alkylating chemotherapy after failure of RT and RT after failure of chemotherapy, patients with wildtype *IDH1* benefitted from RT regardless of *MGMT* status, whereas patients with a methylated *MGMT* promoter showed a larger benefit from chemotherapy. Importantly, patients with *IDH1*-wildtype *MGMT* methylated tumors, who initially received chemotherapy retain their benefit and patients with *IDH1*-wildtype *MGMT* methylated tumors, which initially received RT showed a benefit matching their *MGMT* status (Table e-2). Next we performed a multivariate

analysis, which included all the previously identified prognostic factors from univariate analysis of the NOA-04 trial in addition to *MGMT* and *IDH1*, namely resection status (complete *versus* incomplete *versus* biopsy), histology (astrocytic *versus* oligodendroglial), and age. In the *IDH*-wildtype group, extent of resection, age and histological subtype were prognostic factors. Most importantly, there was an interaction between *MGMT* status and the therapy used; i.e. MGMT promoter methylation predicted benefit from chemotherapy. In contrast, in the IDH-mutated group, there was no interaction between MGMT status and therapy. Only histological subtype remained as a prognostic factor (Figure 1, Table 3). In both groups, there was no prognostic or predictive role for the 1p/19q status (data not shown).

As a next step, we looked at anaplastic glioma patients from the GGN and NOA-08[22] cohorts to confirm our finding. The only prominent difference in baseline characteristics between the RT and the TMZ/RT group was age (Table 1). PFS data from this cohort were plotted separated by the use of alkylator-based treatment (RT vs. alkylator-based chemo- or radiochemotherapy) and *IDH1* mutation as well as *MGMT* promoter methylation status. Patients with *IDH*1 mutation and *MGMT* promoter methylation had a surprisingly long PFS > 5 years for a group of older patients. The small number of patients with the combination of *IDH1* mutation and absence of *MGMT* promoter methylation made formal comparison with the group of patients with *IDH1* mutation and methylated MGMT promoter impossible (Figure e-1a,b, Table 4). In contrast, in patients with *IDH1-w*ildtype tumors *MGMT* promoter methylation was not associated with longer PFS when patients were treated with RT alone (p=0.598), but linked to significantly longer PFS when alkylating chemotherapy was part of the treatment (p=0.018). Analysis for interaction between therapy and *MGMT* status in an analogue Cox-regression model for this validation data set demonstrated a significant interaction term for *IDH1*-wildtype tumors (p=0.039). However, after adjustment to relevant clinical parameters the interaction was not significant any more.

Thus, similar to the anaplastic glioma population of the NOA-04 trial, *MGMT* promoter methylation was only associated with benefit from alkylator-based chemo- and

radiochemotherapy (as compared to RT alone) in patients with *IDH1-w*ildtype anaplastic gliomas (Figure e-1). Interestingly, these results are further supported by an analysis of glioblastoma patients from the GGN cohort. There, a low number of *IDH1* mutated tumors precludes meaningful conclusions for this patient group, but again *MGMT* was predictive for the effect of alkylating chemotherapy in the *IDH1-w*ildtype tumors (Table e-3).

Discussion

Epigenetic silencing of the *MGMT* gene by promoter hypermethylation and the resulting compromise in DNA repair have been associated with longer survival in patients with glioblastoma who receive alkylating agents.[22,23] In the EORTC 26981/22981/NCIC CE.3 glioblastoma trial providing evidence for the use of temozolomide, patients with a hypermethylated *MGMT* promoter preferentially benefited from the addition of TMZ to RT.[1] Similarly, hypermethylation of the *MGMT* promoter was predictive for the response to alkylating agent-based (radio-)chemotherapy in elderly patients. In 233 patients with glioblastoma > 70 years, patients with *MGMT* methylated tumors had longer PFS when treated with RT plus chemotherapy or chemotherapy alone compared to patients treated with RT alone. Patients with *MGMT* unmethylated tumors appeared to derive no benefit from chemotherapy given as primary or salvage treatment.[2] These data were readily confirmed in the randomized NOA-08 trial which resulted in a practice-changing call for routine *MGMT* testing in elderly glioblastoma patients.[20]

NOA-04 challenged the view of a predictive role for *MGMT* promoter hypermethylation in malignant glioma.[3] While NOA-04 confirmed the prognostic relevance of *MGMT* promoter methylation, it did not support the suggestion that *MGMT* promoter methylation is generally predictive for benefit from alkylating chemotherapy.[1] NOA-04 showed a marked difference in PFS between patients with *versus* without *MGMT* promoter methylation who were treated with RT alone, too. This finding was supported by a reanalysis of the EORTC 26951 trial of oligodendroglial anaplastic tumors[5], in which also patients with RT only in the standard arm had a superior PFS, when the *MGMT* promoter was hypermethylated. Thus, in anaplastic gliomas *MGMT* promoter methylation is a favourable prognostic marker independent of the type of therapy, i.e., radio- or chemotherapy. This pattern might be associated with the high incidence of other prognostically favourable molecular markers in these tumors, such as *IDH1* mutation, 1p/19q co-deletion or yet to be identified novel aberrations. It was concluded that *MGMT* promoter hypermethylation in anaplastic gliomas may be regarded as (i) a

prognostic marker for good outcome in patients treated with RT or any type of genotoxic therapy or (ii) predictive for response to RT itself.[3,24]

The R132 mutations in the *IDH1* gene represent the most recent and to date strongest positive outcome marker in anaplastic gliomas that will likely influence histopathological grading as a subclassifier in the group of malignant gliomas as well as stratification in the future trials on anaplastic gliomas and may lead to a better understanding of the differences between anaplastic glioma and glioblastoma.[18] Already the original publication on *IDH1* mutations in glioblastomas had indicated that tumors carrying *IDH1* mutations had a better prognosis than *IDH1-w*ildtype tumors.[25] This has been confirmed across gliomas of WHO grades II-IV, including both astrocytic and oligodendroglial tumors.[3,9,20] In contrast, it has not been possible to link *IDH1* mutations to better responsiveness to specific types of treatment, neither in glioblastoma[12] nor in anaplastic gliomas in the NOA-04[3] or the EORTC 26951 trial.[11] Moreover, in patients with low-grade gliomas *IDH1* mutations were linked to improved overall survival, but not to response to TMZ at progression after RT.[14,26] The frequency of *IDH1* mutation is between 50-70% in WHO grade III, 5-10% in WHO grade IV gliomas of younger patients and almost zero in elderly patients with glioblastoma.[7,24]

The present analysis from the NOA-04 trial suggests an interesting and simple interaction model to explain the discrepancy of the relevance of the *MGMT* status in WHO grade III and IV gliomas. According to our data, *MGMT* promoter methylation is prognostic for patients with *IDH1*-mutant WHO grade III gliomas. In contrast, in patients with *IDH1-w*ildtype tumors, *MGMT* promoter methylation is predictive for benefit from alkylating chemotherapy (Figure 1, Tables 2-4). This finding not only provides a good explanation for a long-standing conflict that proposed a principal difference between WHO grade III and WHO grade IV tumors but also suggests the necessity of testing for both, *IDH1* mutations and methylation status of the *MGMT* promoter. Patients with anaplastic gliomas carrying wildtype *IDH1* and a hypermethylated *MGMT* promoter may not be adequately treated with RT alone, but should be considered candidates for alkylating chemotherapy with TMZ or PCV, or be treated within one of the current trials (e.g. CATNON) for combined radiochemotherapy with TMZ. *IDH1*

mutational status rather than the WHO grade may more precisely determine whether the *MGMT* promoter status is predictive for benefit from alkylating chemotherapy. Interestingly, a similar interaction was not found for 1p/19q and *MGMT* status, but the absence of a 1p/19q co-deletion conferred a worse prognosis in RT- and also chemotherapy-treated patients (data not shown). However, 1p/19q co-deletion has been developed as a strong predictive biomarker by long-term analysis of the EORTC 26951[27] and RTOG 94-02[28] trials and therefore needs to be included into the biomarker panel, which is of immediate relevance for anaplastic glioma patients.

The current analyses were limited by sample and event number to a two-factor interaction term. This is mirroring the clinical situation, where *IDH* information will be available and the decision to test for *MGMT* and to make a treatment decision will follow. In addition to the limited sample sizes, the *post hoc* hypothesis-generating character of this data and the limitations of the supporting data set, the present analysis leaves some questions unanswered. What is the biological basis for the prognostic value of *IDH1* mutations in patients treated with RT? Is there a patient cohort that should be treated with combined radiochemotherapy or may chemotherapy alone be sufficient? Is there a differential role of these biomarkers in one of the histological subgroups? The first question will most likely be answered in the near future in the context of the association between *IDH1* mutations and the so-called GCIMP, where tumors with *IDH1* mutations build a distinct subset of samples displaying concerted hypermethylation at a large number of loci.[29,30] In a subgroup analysis of EORTC 26951, GCIMP status correlated with survival, *MGMT* promoter hypermethylation, 1p/19q co-deletion, and *IDH1* mutation status. GCIMP status strongly increased the predictive accuracy of survival in a model including known clinical prognostic factors such as age and performance score.[31] The strong association between GCIMP status and *MGMT* promoter methylation suggested that the *MGMT* promoter methylation status is part of a more general, prognostically favorable genome-wide methylation profile, which most likely includes radiosensitivity makers. Discovery of these markers may help identifying anaplastic glioma patients that benefit from RT and could further open opportunities for targeted

manipulation of the underlying pathways. Despite evidence for a mere alkylator therapy-predictive role for *MGMT* status from three randomized trials with RT-only and alkylator-based arms[1,20,32], there is also conflicting data from a large retrospective analysis of an MD Anderson cohort. In the latter analysis, there was some prognostic effect of MGMT status for glioblastoma patients treated with RT alone.[33]

The present data are meant to generate an interesting hypothesis and to challenge the way that we are dealing with biomarker information, not marker by marker as in the past and even presently in the EORTC 26951[27] and RTOG 94-02[28] publications, but by acknowledging the interaction of the data that we know of.

The present data from NOA-04, GGN and NOA-08 will provoke a discussion on the standard-of-care arm, RT, in the *IDH1*-wildtype, *MGMT* promoter-hypermethylated patients of the CATNON trial, as well as on the TMZ alone arm in the halted CODEL trial for patients with unmethylated tumors despite the low frequency of 1p/19q codeleted/*MGMT* unmethylated tumors. In these trials, the standard arm is RT and the role of TMZ in patients with anaplastic gliomas without 1p/19q codeletion (CATNON) or with the codeletion (CODEL) is investigated. Similar, there will be a discussion on the standard-of-practise also outside trials. Data from these trials may further validate the role of *MGMT* as a predictive biomarker in the *IDH1-w*ildtype patient population. It may confirm that alkylating chemotherapy produces no benefit in patients with unmethylated, *IDH1-w*ildtype tumors, but will provoke the question whether TMZ alone with deferred RT may be a sufficient treatment in patients with *MGMT* promoter methylated and *IDH1-w*ildtype tumours.

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Figure Legend

Figure: Kaplan-Meier survival estimates in the NOA-04 biomarker cohort. PFS is shown by *IDH1* mutation status (mutated or wildtype) and *MGMT* promoter methylation status (*MGMT* promoter methylated (*MGMT*+) or unmethylated (*MGMT*-) for RT-treated patients (blue lines) or chemotherapy-treated patients (red lines**).** In this cohort 25 events were censored in the RT and 16 in the chemotherapy groups, respectively. Vertical lines on the Kaplan-Meier curves indicate this.

Tables

Table 1. Baseline Patient Characteristics

	NOA-04 training cohort		NOA-08/GGN validation cohort	
	RT (n = 91)	**PCV or TMZ (n = 92)**	**RT (n =42)**	**TMZ ± RT (n =67)**
Median age (range), years	44 (23-74)	42 (20-77)	67 (23-75)	50 (23-80)
Central histopathology, n				
Anaplastic astrocytoma	40	41	32	45
Anaplastic oligoastrocytoma	34	38	9	19
Anaplasticoligodendroglioma	17	13	1	3
Median KPS (range) [%]	100 (70-100)	100 (70-100)	90 (80-100)	90 (50-100)
Unknown, n			6	5
Resection, n				
Total	39	32	15	29
No total	43	47	21	33
Biopsy	9	13	5	4
Unknown			1	1
Co-deletion of 1p/19q, n				
Yes	36	32	NA	NA
No	46	48		
Missing	9	12		
MGMT promoter, n				
methylated	55	61	31	47
unmethylated	36	31	11	20
IDH1, n				
wildtype	30	30	24	33
mutant	61	62	18	34

Abbreviations: German Glioma Network (GGN), isocitrate dehydrogenase (IDH), Karnofsky Performance Score (KPS), O6-methyl-guanyl methyltransferase (MGMT), not available (NA), procarbazine/CCNU/vincristine (PCV), radiotherapy (RT)
(Tests for homogeneity: Age and KPS: Mann-Whitney-U-Test; other variables: Fisher-Exact-Test)
*There were no *IDH2* mutations in the samples examined from the NOA-04 cohort.
Baseline characteristics differed significantly (p=0.027) for median age in the GGN/NOA-08 validation cohort .

Table 2. Progression-free survival NOA-04 Biomarker Cohort					
		RT		**Chemotherapy**	
		Median (95% CI), months	**N**	**Median (95% CI), months**	**N**
IDH1-mutant	MGMT methylated	36.8 (34.4-NR)	45	44.7 (34.7 - NR)	47
	MGMT unmethylated	28.0 (10.9 - NR)	16	28.1 (7.4 – NR)	15
IDH1-wildtype	MGMT methylated	16.3 (4.9 – 23.6)*	10	**27.2 (8.7 – 50.0)****	14
	MGMT unmethylated	17.2 (9.6 – 34.2)*	20	**9.1 (6.8 – 17.1)****	16

Abbreviations: confidence interval (CI), isocitrate dehydrogenase (IDH), O6-methyl-guanyl methyltransferase (MGMT), not reached (NR), radiotherapy (RT)

*LogRank-test for methylation effect (RT- *IDH1*-wildtype), p=0.331
**LogRank-test for methylation effect (chemotherapy), p=0.016

Table 3. Prognostic Model for the NOA-04 Biomarker Cohort				
NOA-04 Biomarker Cohort *IDH*-wildtype (n=60, 51 with progression)		**Cox regression**		
Variable (Risk)		**Hazard ratio**	**95%-CI**	**P**
Progression-free survival				
Biopsy or incomplete vs. complete resection		2.35	1.24-4.44	0.009
Age in yrs.		1.04	1.01-1.06	0.004
Astrocytoma vs. oligodendroglial tumor		3.63	1.74-7.55	0.001
Chemotherapy vs. RT		3.02	1.40-6.49	0.005
MGMT methylated vs. unmethylated		2.02	0.84-4.89	0.117
Interaction *MGMT**Therapy		0.11	0.03-0.40	0.001
NOA-04 Biomarker Cohort *IDH*-mutated (n=123, 58 with progression)		**Cox regression**		
Variable (Risk)		**Hazard ratio**	**95%-CI**	**P**
Progression-free survival				
Biopsy or incomplete vs. complete resection		1.14	0.65-1.99	0.658
Age in yrs.		1.01	0.99-1.04	0.401
Astrocytoma vs. oligodendroglial tumor		2.50	1.39-4.49	0.002
Chemotherapy vs. RT		1.34	0.52-3.44	0.540
MGMT methylated vs. unmethylated		0.66	0.29-1.52	0.334
Interaction *MGMT**Therapy		0.80	0.26-2.47	0.697

Abbreviations: confidence interval (CI), *isocitrate dehydrogenase* (*IDH*), Neurooncology Working Group of the German Cancer Society (NOA), *O6-methyl-guanyl methyltransferase* (*MGMT*), radiotherapy (RT)

Prognostic factors in the NOA-04 trial were: age, extent of resection, 1p/19q status, *MGMT* status and *IDH* status.[3] In the present analysis of the biomarker cohort, the factor extent of resection was no longer prognostic in both arms and age as well as histological subtype are no longer prognostic in the RT arm of this biomarker subset of patients.

Table 4. Progression-free survival in the GGN Anaplastic Astrocytoma / NOA-08 Cohort					
		RT		**Chemotherapy ± RT**	
		Median (95%-CI), months	**N**	**Median (95%-CI), months**	**N**
IDH1-mutant	*MGMT* methylated	71.5 (48.3-94.8)	17	56.5 (34.2-78.9)	32
	MGMT unmetylated	-	1	-	2
IDH1-wildtype	*MGMT* methylated	5.3 (3.7-6.8)*	14	**15.8 (2.5-29.1)****	15
	MGMT unmethylated	9.3 (5.2-13.4)*	10	**3.4 (2.0-4.8)****	18

Abbreviations: confidence interval (CI), German Glioma Network (GGN), *isocitrate dehydrogenase* (*IDH*), Neurooncology Working Group of the German Cancer Society (NOA), *O6-methyl-guanyl methyltransferase* (*MGMT*), radiotherapy (RT)

*LogRank-test for difference: p=0.598
**LogRank-test for difference: p=0.018

Supplementary Information on

Prognostic or predictive value of *MGMT* promoter methylation in gliomas depends on *IDH1* mutation

Wolfgang Wick, MD[1,3], Christoph Meisner, PhD[5], Bettina Hentschel, PhD[6], Michael Platten, MD[1], Alissa Schilling, MD[1], Benedikt Wiestler, MD[1,3], Michael C. Sabel, MD[7], Susanne Koeppen, MD[10], Ralf Ketter, MD[11], Markus Weiler, MD[1,3], Ghazaleh Tabatabai, MD, PhD[12], Andreas von Deimling, MD[2,4], Dorothee Gramatzki, MD[12], Manfred Westphal, MD[13], Gabriele Schackert, MD[14,15], Markus Loeffler, MD[6], Matthias Simon, MD[16], Guido Reifenberger, MD[8,9], Michael Weller, MD[12]

Supplementary Tables

Table e-1: TTF and PFS

NOA-04 cohort	***Per Protocol*** **Radiotherapy (n = 139)**	***Biomarker*** **Radiotherapy (n=91)**	***Per Protocol*** **PCV or temozolomide (n = 135)**	***Biomarker*** **PCV or temozolomide (n=92)**
Median time-to-treatment failure, months (95%-CI)	40.4+	42.7+	43.8 (37.4-NR)	43.5 (33.0-NR)
Astrocytoma	32.0 (23.3-NR)	32.9 (23.8-NR)	29.4 (19.0-42.0)	21.6 (13.5-37.4)
Oligoastrocytoma	NR	42.7+	NR	NR
Oligodendroglioma	NR	NR	52.6 (29.8-NR)	52.6 (29.8-NR)
Treatment failure at 48 months, % (95% CI)	55.5 (46.3-64.6)	59.8 (48.7-70.9)	46.4 (36.7-56.1)	46.7 (35.5-58.0)
Median progression-free survival, months (95%-CI)	30.6 (16.3-42.8)	34.2 (18.4-47.6)	31.9 (21.1-37.3)	31.2 (19.6-39.1)
Astrocytoma	10.8 (8.9-28.3)	16.3 (8.9-30.6)	18.2 (12.1-24.2)	15.7 (8.7-20.6)
Oligoastrocytoma	52.1 (18.4-NR)	51.0 (14.9-NR)	52.7 (32.8-NR)	50.0 (31.9-NR)
Oligodendroglioma	47.6 (34.6-NR)	47.6 (25.7-NR)	21.4+	33.9 (12.0-NR)

Abbreviations: confidence interval (CI), not reached (NR), procarbazine/CCNU/vincristine (PCV), progression-free survival (PFS), time-to-treatment failure (TTF), radiotherapy (RT)

Table e-2. Time-to-treatment failure in the NOA-04 Biomarker Cohort					
		RT		**Chemotherapy**	
		Median (95% CI), months	**N**	**Median (95% CI), months**	**N**
IDH1-mutant	*MGMT* methylated	>54 (45.4-NR)	45	49.7 (43.7 - NR)	47
	MGMT unmethylated	>54 (NR - NR)	16	>54 (47.4 – NR)	15
IDH1-wildtype	*MGMT* methylated	33.3 (28.9 – 38.1)	10	35.8 (20.7 – 52.0)	14
	MGMT unmethylated	25.2 (16.6 – 34.9)	20	16.1 (10.8 – 20.5)	16

Abbreviation: confidence interval (CI), not reached (NR), radiotherapy (RT)

Table e-3. Progression-free survival in the GGN Glioblastoma Cohort					
		RT		**Chemotherapy ± RT**	
		Median (95%-CI), months	**N**	**Median (95%-CI), months**	**N**
IDH1-mutant	*MGMT* methylated	18.1 (0-44.8)	7	31.7 (18.3-45.1)	13
	MGMT unmetylated	-	1	12.8 (2.2-23.5)	5
IDH1-wildtype	*MGMT* methylated	5.5 (3.4-7.6)	28	**10.0 (5.1-14.8)**	87
	MGMT unmethylated	6.2 (5.8-6.6)	38	**6.1 (5.1-7.1)**	109

Abbreviations: confidence interval (CI), radiotherapy (RT)

Supplementay Figures

Figure e-1a – Radiotherapy

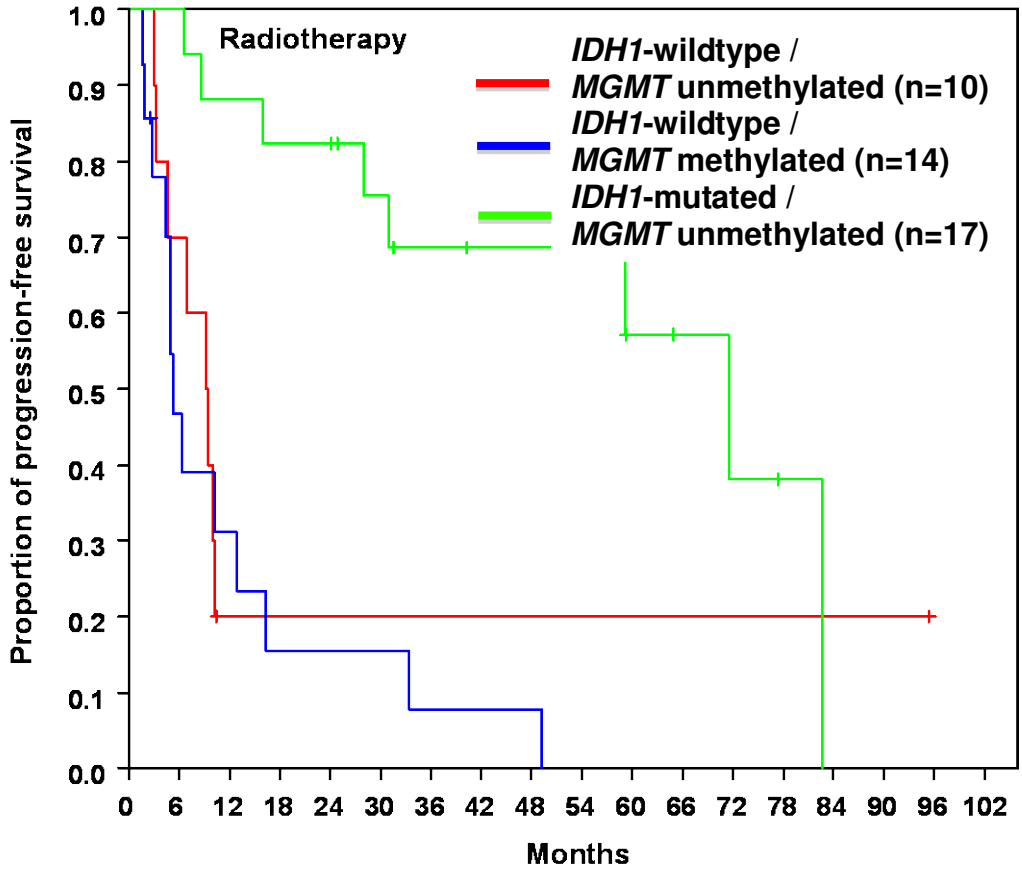


Figure e-1b – Alkylating chemotherapy with or without radiotherapy

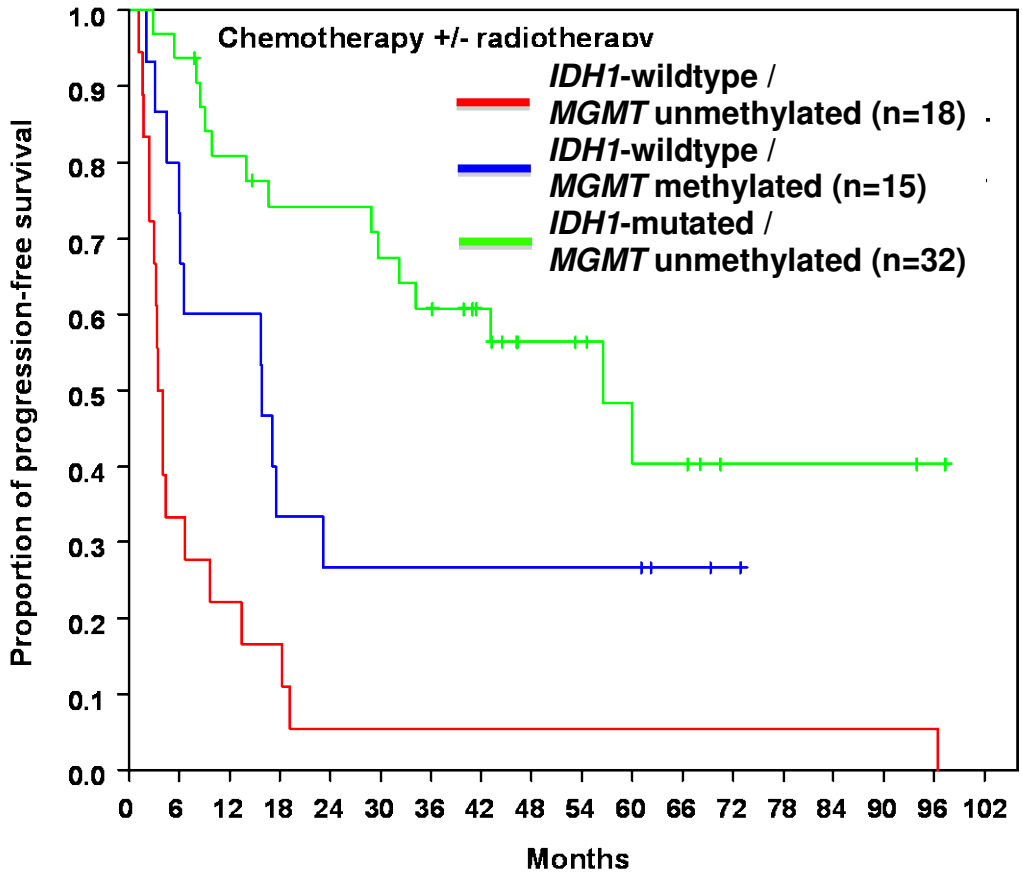
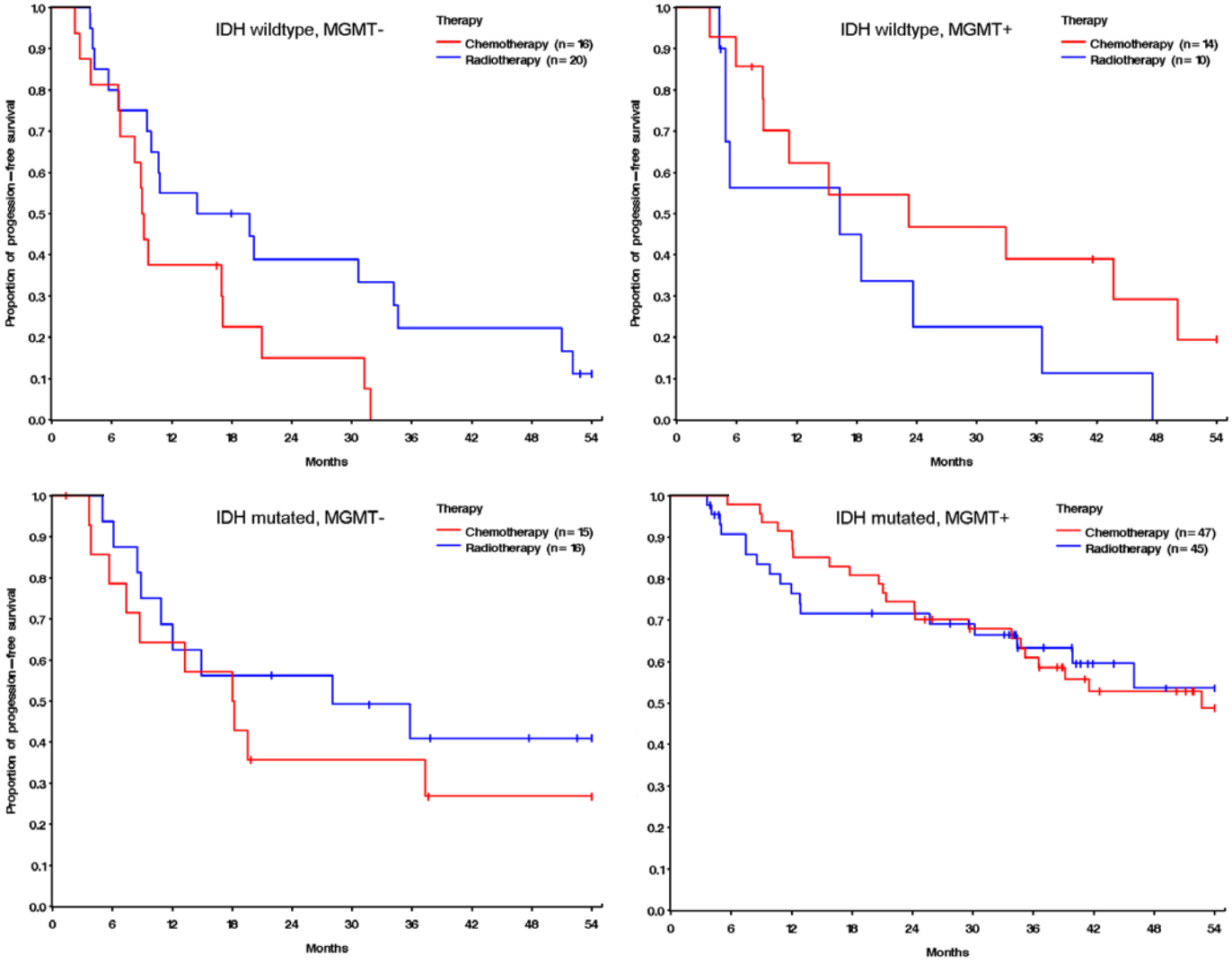


Figure Legends

Figure e-1: Kaplan-Meier survival estimates for the anaplastic astrocytoma cohort from the pooled NOA-08/GGN cohorts. PFS data plotted by *IDH1* mutation status (mutated or wildtype) and *MGMT* promoter methylation status (*MGMT* promoter methylated (*MGMT*+) or unmethylated (*MGMT*-) for patients treated with RT (panel a) or chemotherapy/radiochemotherapy (panel b). One patient with RT and *IDH1* mutation and no *MGMT* promoter methylation had PD after twenty months. This curve is not shown in panel a. In panel b the curve for two patients with *IDH1* mutation and no *MGMT* promoter methylation is also not shown (PD after 50 months and censored time after 74 months). In this cohort 11 events were censored in the RT and 19 in the RT ± chemotherapy groups, respectively. This is indicated by vertical lines on the Kaplan-Meier curves.

Figure 1


IDH wildtype, MGMT-
Therapy
Chemotherapy (n= 16)
Radiotherapy (n= 20)
Proportion of progression−free survival
Months
IDH wildtype, MGMT+
Therapy
Chemotherapy (n= 14)
Radiotherapy (n= 10)
Proportion of progression−free survival
Months
IDH mutated, MGMT-
Therapy
Chemotherapy (n= 15)
Radiotherapy (n= 16)
Proportion of progression−free survival
Months
IDH mutated, MGMT+
Therapy
Chemotherapy (n= 47)
Radiotherapy (n= 45)
Months